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CHAPTER 20

Physiological Characteristics and the Species Concept in *Actinomycetales*

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Physiological characteristics play a dominant role in characterization, classification, and speciation of procaryotes. Because of limited morphological diversity, conventional systematics of these microorganisms still is in a disappointing state. Fortunately, with many *Actinomycetales* morphological diversity does occur. This diversity can be used to advantage in systematics because it represents the result of a complex multitude of "physiological reactions" expressed in the phenotypic criterion of morphology. Morphological information coupled with that from more sophisticated approaches to characterization and classification, e.g., cell-wall analyses, determination of guanine and cytosine ratios in nucleic acids, nucleic acid hybridizations, and genetic studies, would seem to offer the best contemporary approach to assessing the significance of "physiological characteristics" as they now are used. Physiological characteristics represent responses of the organism to various aspects of the environment, are based on enzymatic activities, and are subject to natural variation and induced mutation. Many physiological reactions, as expressed in the literature on systematics of procaryotes, represent multiple enzymatic activities. There is need to clarify this aspect of conventional procaryote systematics to determine the real significance of such reactions to systematics and speciation.

INTRODUCTION

"Physiological" or "biochemical" characterization of microorganisms in connection with classification and identification of strains has occupied a dominant position in systematics since procaryotes and eucaryotes first were studied. Interpretation of such characterization data also has been important in development of species concepts. First reports embracing physiological characteristics understandably were of a simple nature, e.g., determination of the abilities of microorganisms to grow on a variety of natural substrates. Many of these reports were lengthy and very subjective (Rossi Doria 1891). Unfortunately, some modern reports could be categorized in the same manner. Since the beginnings of bacteriology, a legion of characterization tests has been devised and studied. Some of these tests have enjoyed popularity because they allowed categorization of strains and a means for communicating identifications. Many ultimately met with disfavor and were abandoned because study of additional strains led to detection of the inevitable "different" strain or strains whose characteristics did not seem to correlate properly with other data or an overwhelming number of strains gave similar results and hoped for separations could not be made. Today, bacteriologists and zymologists still are confronted with many such physiological reactions and tests. Much gadgetry has been developed, particularly for studying and identifying bacteria of medical importance where time and accuracy of identification are of essence.

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

There seems to be little question that many methods for identification based on physiological reactions are of contemporary value. However, their significance in developing reasonable, valid, and acceptable species concepts remains obscure.

Mycologists, in general, with the exception of zymologists, seem to have avoided much degree of reliance on physiological reactions of the kind emphasized by bacteriologists and zymologists. They have avoided the "Physiological trap" wherein study of increasing numbers of strains of a particular taxon turns up "physiological variants." However, with increasing exploitation of molds, there will be increasing development of tests for separation and identity based on physiological or biochemical reactions. Mycologists will be faced with problems similar to those confronting bacteriologists and zymologists. On the other hand, mycology and zymology are favored by the presence of sexual stages exhibited with more definition in their microorganisms than the sexual stages associated with bacteria. This phenomenon is less readily defined with the procaryotes including the *Actinomycetales*. Because of evolutionary impact, there is no precise definition of a species of *Actinomycetales* in any of the genera embraced within the Order; there may never be. But, there can be developed more precise concepts that will facilitate our understanding of these microorganisms and their role in nature. The sophisticated technology available today suggests that more precision can be imparted to characterizations of microorganisms.

Much success has been achieved in relatively precise characterization of genera of *Actinomycetales* through morphological studies and collation of such information with compositional analyses. Even more precision is being achieved as the determination of moles percent guanine and cytosine and degree of DNA/DNA and DNA/RNA hybridization become more routinely applied. Cell-wall analyses and molecular biological studies represent a higher degree of sophistication and precision than conventional systematics heretofore has enjoyed. The purpose of this paper is to suggest ways in which conventional physiological reactions can be elevated to a similar plane of sophistication. Hopefully, that would give even greater precision to characterization, a better view of relationships of strains and taxa, and better bases for determining similarities. Also, for genetic studies, such more precise data would seem warranted. From all that, perhaps more valid and more clearly defined species concepts can emerge.

Discussion

Conventional Systematics Applied to the Actinomycetales

Physiological or biochemical characteristics of microorganisms have been plaguing bacteriologists for many years whether it has been realized or not. Such characteristics have played a dominant role in characterization, classification, and speciation of procaryotes. Necessarily, they have been resorted to because of limited morphological diversity in these microorganisms. Many such characteristics have enjoyed popularity because their general natures seemed intriguing. Results seemed to have some degree of precision and seemed to allow *separation* of some strains and categorization into understandable "groups" or categories.

The group of bacteria more properly known as the *Actinomycetales* occupies a unique position because some degree of morphological diversity does occur among its members. This diversity has been used to advantage in establishing generic identities. There seems to be general agreement that morphological diversity represents the result of a complex multitude of "physiological reactions" expressed in the phenotypic criterion of morphology. From that point on, there have been many attempts to utilize physiological reactions for characterization, classification, and identification. The disappointing state of conventional systematics of

TABLE 1. *Physiological reactions used in preparing descriptions of "species" of* Actinomycetales *in the 1957 and 1974 editions of* Bergey's Manual of Determinative Bacteriology

Genus	No. of Physiological Reactions Selected[a]	Specific Enzyme Indicated	No. of Physiological Reactions Selected	Specific Enzyme Indicated
	Bergey 1957		Bergey 1974	
Actinomyces	20	None	> 20	Urease, catalase
Arachnia			46	Urease, catalase
Bifidobacterium			59	Fructose-1,6-diphosphate aldolase Glucose-6-phosphate dehydrogenase Phosphoketolase Transaldolase Catalase 6-Phosphogluconate dehydrogenase Fructose-1,6-diphosphate dehydrogenase
Actinoplanes	None	None	12	None
Bacterionema			70	Catalase, urease, acid phosphatase
Nocardia	65	Catalase	101	Catalase
Micromonospora	8	None	55	Catalase, urease
Thermoactinomyces	6	None	14	None
Streptosporangium	None	None	29	None
Streptomyces	65	Catalase, tyrosinase	37	20-β-Keto reductase, α-galactosidase
Rothia			51	Catalase, urease
Mycobacterium			86	Catalase, urease, amidase, α-esterase, β-esterase, Isonicotinamidase, Allantoinase, Succinamidase, Propionamidase, Nicotinamidase, Pyrazinamidase, Arylsulfatase, Benzamidase, Acid Phosphatase
Frankia			15	None
Spirillospora			5	None
Amorphosporangium			5	None
Ampullariella			5	None
Pilimelia			6	None
Planomonospora			30	None
Planobispora			23	None
Dactylosporangium			26	None
Kitasatoa			8	None
Dermatophilus			24	Catalase, urease
Geodermatophilus			20	None
Pseudonocardia			8	None
Streptoverticillium			19	None
Sporichthya			21	None
Microellobosporia			21	None
Micromonospora			55	Catalase, urease
Actinobifida			12	None
Thermomonospora			12	None
Microbispora			17	None
Micropolyspora			13	None
Promicromonospora			29	None
Actinomonospora			29	None

[a]Based on rapid scans of each species description included in 1957 and 1974 *Bergey's Manuals*. Numbers should be considered to be only relative.

these microorganisms is, in part, due to these attempts. Trejo (1970), Luedemann (1971), and Pridham (1976a) have discussed problems, among others, with physiological reactions in study of *Actinomycetales*.

Chronicling, or setting down the histories of strains and designating them, can be done in reasonably understandable and communicable fashion. Characterization of progeny of strains is less reproducible, less understandable, and less communicable. Major obstacles are the availability of numerous tests and methodologies and their standardization. Beyond that, the reproducibility of conventional characterizations and, more importantly, their interpretation leaves much to be desired.

The descriptions of "species and subspecies" included in the 1957 and 1974 editions of *Bergey's Manual of Determinative Bacteriology* (Breed et al. 1957; Buchanan and Gibbons 1974) for different kinds of physiological reactions and specific enzyme activities give the information listed in Table 1.

From examination of the two volumes of *Bergey's Manual* it is apparent that little standardization of test methods prevails. The same is true for most other systematic treatises. Gottlieb (1976) has addressed this problem to some extent in a recent letter to the editor of the *Newsletter of the U.S. Federation for Culture Collections*. Even within the same genus of *Actinomycetales*, comparative data are lacking. For example, information on production of 20-β-keto reductase was presented for only one "species" of streptomycete. This immediately raises the question, "Do any, or all, of the other 'species' described produce this enzyme? What is its real significance?" Catalase and urease were the most often reported specific enzymes for *some* species of *some* genera. What about the catalase- and urease-producing potential of all the others? Much is left to inference. It would seem to be the obligation of any investigator developing a new "physiological test" to study the test with progeny of the holotype strain of each "species" within the genus under study. In our laboratory we have been spending much time and effort trying to complete information for some of the simpler and more routine physiological reactions missing from descriptive information published on streptomycetes and streptoverticillia. Ultimately, this information will allow better comparisons and a clearer understanding of relationships of strains. Clearly, there is a degree of irresponsibility to the scientific community evidenced by the present gaps in information.

Table 2 lists a few representative physiological criteria that have been used for characterization of *Actinomycetales*. Some of these criteria undoubtedly will be abandoned because they are no longer precise enough to fit contemporary needs for reproducibility, precision, and relevance to contemporary species concepts.

TABLE 2. *Some physiological reactions used to characterize* Actinomycetales (Bergey's Manual 1974)

Action on gelatin
Action on gelatin
Utilization of carbohydrates
Fermentation of carbohydrates
Utilization of salts of organic acids
Sensitivity to antibiotics and other compounds
Formation of pigments
Sodium chloride tolerance
Production of secondary metabolites
Production of primary metabolites

Modern Methods of Classification

Progress made during the past few years in determination and categorization of morphology of *Actinomycetales*, coupled with information obtained by more sophisticated approaches to characterization and classification, e.g., cell-wall analyses (Lechevalier and Lechevalier 1976), determination of guanine and cytosine ratios in nucleic acids and nucleic acid hybridizations (Bradley and Huitron 1973; Woodruff et al. 1973; Bradley 1975), and genetic studies (Hopwood 1973), would seem to offer the best contemporary approach to assessing the significance of physiological characteristics as they now are used. There now are available more precisely defined groups of *Actinomycetales*, i.e., the genera, because of these developments. An increasing recognition or understanding of the holotype strain concept should allow even better understanding of broad relationships at the generic level utilizing the criteria mentioned above. Also, recognition of the "wild type" concept continually must be kept in mind. One begins to wonder, however, what a "wild type" might be in view of the increasing environmental stresses that are occurring in nature. Certainly, progeny of the holotype strain or the officially designated neotype strain of each species of each genus of *Actinomycetales* should be subjected to study based on the criteria mentioned above. As an alternative, when neither of these types is available, a carefully selected reference strain readily available to all should be used. Much time and energy are involved in pursuit of such a goal. Obstacles to obtaining more precise characterizations include equipment costs and availability and technological problems. Because of these obstacles, the simpler kinds of physiological characteristics and their determinations still represent inviting objects for study. Such an approach, however, would not seem appropriate in view of contemporary technology available.

Use of Multiple Enzyme Activities

Physiological characteristics represent responses of the organism to various aspects of the environment, are based on enzymatic activities, and are subject to natural variation and induced mutation. Many of the physiological characteristics or reactions, as listed in Tables 1 and 2 and expressed in the literature on systematics of procaryotes, represent multiple enzymatic activities. A glance at any of the metabolic charts or texts on enzymology now available (Lehninger 1970; Sallach 1972; Michal 1974) will quickly confirm the multiple enzyme aspects. Multiple enzyme aspects, in turn, should bring to mind the one gene-one enzyme concept and its more modern interpretations, e.g., one gene-one polypeptide. Involved also is the rapidly developing area of isozyme study (Markert 1975). Many of the physiological characteristics now in vogue for characterizing *Actinomycetales* represent catabolic metabolism, e.g., liquefaction of gelatin, urease production and utilization, assimilation, or fermentation of many kinds of compounds. Perhaps considering these reactions in terms of their enzymatic equivalents and developing or modifying methods for enzyme detection and quantitation could raise the nature of present tests to a more sophisticated plane. Study of the elaboration of specific enzymes and isozymes would provide more sophisticated and precise data on physiological characteristics. This approach has been described as the comparative analysis of control patterns for enzymes by Rehacek (1973). Such information could result in the elimination of some tests which, on an enzymatic basis, may be measuring the same reaction and might help to resolve some of the "weighting" problems that come to mind in consideration of computer assessment of systematic data. Also, such information would provide a better basis for genetic studies. Understandably, there are difficulties for conventional systematists moving to this more advanced technological

stage, e.g., problems of constitutive and inducible, repressed or derepressed enzymes, problems with extractions of large numbers of strains, as well as problems concerned with actual detection of enzymes. Alternatively, study could be made of the enzymes controlling anabolic processes such as biosynthesis of deoxyribonucleic acid, ribonucleic acid, purines, and pyrimidines.

Computer Assessment

If we continue to pursue the conventional approach, the numbers of kinds of such tests that could be examined are infinite. Rogosa et al. (1971) have compiled a large listing of such kinds of tests in their publication on coding data for computer assessment. Some specific enzymes are mentioned therein. Many of the physiological reactions listed obviously are based on multiple enzymatic activities, hence multiple genes. It would seem that the use of computer assessment represents the most effective way to handle the results obtained in the conventional way. With the *Actinomycetales* there have been a number of such studies wherein large numbers of features (many of them physiological characteristics) are utilized as shown in Table 3.

TABLE 3. *Computer assessment of characteristics of* Actinomycetales

Organisms	No. of Strains Studied	No. of Features Entered	Reference
Nocardiae	112	100	Tsukumura 1969
Actinomycetales	17	70	Davis and Newton 1969
Mostly streptomycetes	63	91	Gilardi et al. 1960
Mostly streptomycetes	63	91	Hill et al. 1961
Mostly streptomycetes	190	108	Silvestri et al. 1962
Actinomycetales	7	123	Goodfellow 1967
Mycobacteria	600-700	30	Wayne 1967
Actinomycetales	5	98	Skyring and Quadling 1969
Streptomycetes	2557	76	Szabo et al. 1967
Nocardioform bacteria	28	241	Goodfellow 1971

One does wonder what results would be obtained with a series of several hundred mutant and/or variant strains from the same "wild type" parent, progeny of which were subjected to the conventional physiological characterizations and the results assessed by computer. Inclusion of progeny of the holotype strains of several variously named *Actinomycetales* would give added interest to such a study.

Likewise, one wonders whether differences might become apparent if some kind of weighting were done based on numbers and kinds of enzymes involved in particular physiological reactions now presumably given a weight of "one." Many of these features represent multiple enzyme activities. One wonders whether any differences might result in assessment if these were considered in this light.

There is need to clarify the "physiological reaction" aspect of conventional procaryote systematics to determine its real significance to systematics and to speciation. Gelatin liquefaction, casein digestion, and disaccharide utilization all have been stated to be of minor metabolic significance and subject to single gene mutation (Lamanna and Malette 1965). Nevertheless, these physiological reactions still are in use and still have a great deal of significance attached to them by some conventional systematists.

Characterization by Carbon Assimilation Patterns

Much attention has been focused on utilization of carbon compounds in chemically defined media under standardized conditions for characterization, classification, speciation, and identification of streptomycetes and streptoverticillia. As with many physiological characteristics, the results appeared promising in initial studies because relatively few strains were studied. With time, it has become increasingly difficult to utilize this aid because many seemingly different strains give essentially similar patterns. Studies on natural variation clearly lead to differences in utilization patterns. One has only to examine the many papers on which mutants and variants of *Steptomyces aureofaciens* Duggar have been described. There is no question that variation occurs with many if not all the physiological characteristics discussed. In final analysis, setting more precision to determination of such characteristics will aid in refining species concepts and in relating them to the dynamic situation that exists in nature. The dynamic state, coupled with natural variation and probable induced mutation based on the presence now of many chemicals of one kind or another in habitats, has led us to develop a four-stage system for identification of strains of streptomycetes and streptoverticillia (Pridham 1976b). "Physiological reactions" in the usual sense have been relegated less priority for speciation in this system.

Secondary Metabolite Identification

Actinomycetologists should intensify their efforts at a higher plane of sophistication to arrive at better understanding of physiological reactions and to collate this information with genetic data. A colleague has commented that production of secondary metabolites is variable between strains and one problem is that of detecting traces of product. One would expect the same problem with regard to detection of specific enzymes in biosynthetic paths leading to trace amounts of metabolite. It is far easier to detect metabolites than to measure enzyme activities. Problems include expensive substrata and difficulties in extraction and in measurement of enzymes. He goes on to suggest that contemporary instrumentation, e.g., gas-liquid chromatography (GLC), mass spectrum (MS) determination, and nuclear magnetic resonance (NMR) studies lend themselves far better to establishment of new physiological tests based on metabolic end products than to detection of complex enzymic activities.

At the present time, I have concluded that the most precise handle we have for characterizing and assessing similarities of strains is the qualitative identification of secondary metabolites such as the antibiotics. This criterion seems promising because, like morphology, it is the result of numerous enzymatic activities operating simultaneously or consecutively (Hash 1975). The technical difficulties associated with this criterion seem overwhelming, but not insurmountable, for the conventional systematist.

Specific Enzyme Identification for Actinomycetales

An alternative approach would be that of precise study of enzymes and isozymes involved in catabolism and anabolism of these organisms. Recent literature on *Actinomycetales* provides much information on formation of specific enzymes, and there is indication that the "antibiotics era" is being supplanted by an "enzyme era." Associated with this change, there seems to be increasing industrial and medical utilization of enzymes and enzyme inhibitors and development of immobilized enzyme technology. Table 4 lists a few such enzymes and their sources.

Information available suggests development of a battery of single enzyme tests carried out with chemically defined substrata under standardized conditions such as elaborated in the

TABLE 4. *Some enzymes formed by* Actinomycetales

Enzyme	Source	Reference
UDPGlcUA carboxy lyase	*Ampullariella digitata*	Fan and Feingold 1972
δ-Aminolevulinic acid dehydratase	*Streptomyces olivaceus*	Sato et al. 1971
RNA polymerase (DNA dependent)	*Streptomyces aureofaciens*	Malikova et al. 1972
Lecithinase	Streptomycetes and streptoverticillia	Nitsch and Kutzner 1969
α-D-Mannosidase	*Streptomyces griseus*	Inamine and Demain 1970
Urease	*Actinoplanes* sp.	Sears 1971
RNAase	*Streptomyces* sp.	Abrosimova-Amel'yanchik et al. 1972
Kinonases AI and AIII	*Streptomyces kinoluteus*	Nakamura et al. 1969
Chorismate mutase	*Streptomyces venezuelae*	Görisch and Lingens
Phenoxazinone synthetase	*Streptomyces antibioticus*	Jones and Weissbach 1970
L-Asparaginase	*Streptomyces griseus*	DeJong 1972
β-Amylase	Streptomycetes	Hayashibara Co. 1970
N-Acetyl-muramidase	*Streptomyces albus*	Heymer et al. 1970
Acetamidase	*Nocardia asteroides*	Berd 1973
Adenyl cyclase	*Nocardia erythropolis*	Ide 1971

many volumes of *Methods in Enzymology* (Hash 1975) and similar works. Such a battery of tests might be amenable to automation as now used in some medical laboratories. Certainly, more specific and precise information would be provided for computer assessment of strain similarities. Present characterization technology undoubtedly would be raised to a higher plane of sophistication.

TABLE 5. *Some enzymes involved in pyrimidine (cytosine, thymine), purine (adenine, guanine), deoxyribonucleic acid, and ribonucleic acid biosynthesis*[a]

Pyrimidine biosynthesis
- Carbonyl phosphate synthetase
- Aspartate transcarbamylase
- Orotidine-5'-decarboxylase
- Deoxythymidine 5'-phosphate kinase
- Nucleoside diphosphate kinase

Purine biosynthesis
- Phosphoribosylpyrophosphate amidotransferase
- Adenylosuccinase
- Inosinicase
- Nucleoside diphosphate kinase
- Ribonucleotide reductase

Deoxyribonucleic acid (DNA) biosynthesis
- DNA polymerase
- DNA ligase
- Endonucleases

Ribonucleic acid (RNA) biosynthesis
- Met-tRNA transformylase
- Aminoacyl tRNA synthetases
- tRNA transmethylase
- RNA polymerase
- Deformylase

[a]Taken from Sallach (1972).

Species concepts of each genus of the Order *Actinomycetales* (Buchanan and Gibbons 1974) continue to depend upon individualist treatment as given by the specialists reporting. These concepts based on the conventional systematics of the past possibly may be brought to better agreement through closer approaches to gene complex characterization by developing and adopting such more precise and sophisticated methodologies.

Comparative studies of enzyme systems involved in purine, pyrimidine, deoxyribonucleic acid, and ribonucleic acid biosynthesis offer intriguing possibilities. Provided the proper test strains are used, i.e., the holotype strains of each genus in the *Actinomycetales*, the results would be most interesting. Some of the enzymes involved in these biosyntheses are listed in Table 5.

Conclusions

In summary, conventional systematics of *Actinomycetales* still is in a disappointing state because of much reliance on imprecise methodology for determining physiological characteristics. This, in turn, affects the nature of species concepts derived therefrom. There is need to implement characterizations of these microorganisms along more precise and sophisticated lines. Intensive and deliberate comparative study of control patterns for enzymes and isozymes, particularly those involved in purine, pyrimidine, deoxyribonucleic acid, and ribonucleic acid biosynthesis, offers intriguing possibilities in this direction. Study of these and other enzyme systems would impart more precision and sophistication to the systematics of these microorganisms. Technical difficulties and the large number of holotype strains which should be examined seem overwhelming, but such study would much improve our basis for considering the nature of the *Actinomycetales* and speculating on phylogeny and species concepts.

Literature Cited

Abrosimova-Amel'yanchik, N.M., O. I. Artamonova, R. I. Tatarskaya, and A. A. Baev. 1972. RNase from *Actinomyces* sp., strain 236, forming fragments with a terminal 5'-phosphate (nuclease A236). *Dokl. Akad. Nauk. S.S.S.R.* 207(4):985-987; *Microbiol. Abstr.* 8B(4):57-58. Abstract 8B2854 (1973).

Berd, D. 1973. *Nocardia asteroides*. A taxonomic study with clinical correlations. *Am. Rev. Respir. Dis.* 108:909-917.

Bradley, S. G. 1975. Significance of nucleic acid hybridization to systematics of Actinomycetes. *Adv. Appl. Microbiol.* 19:59-70.

Bradley, S. G., and M. E. Huitron. 1973. Genetic homologies among nocardiae. Chapter 24. *Dev. Ind. Microbiol.* 14:189-199.

Breed, R. S., E. G. D. Murray, and N. R. Smith, eds. 1957. *Bergey's Manual of Determinative Bacteriology*, pp. 694-829. 7th ed., The Williams & Wilkins Co., Baltimore.

Buchanan, R. E., and N. E. Gibbons, eds. 1974. *Bergey's Manual of Determinative Bacteriology*, pp. 657-865. 8th ed., The Williams & Wilkins Co., Baltimore.

Davis, G. H. G., and K. G. G. Newton. 1969. Numerical taxonomy of some named coryneform bacteria. *J. Gen. Microbiol.* 56:195-214.

DeJong, P. J. 1972. L-Asparaginase production by *Streptomyces griseus*. *Appl. Microbiol.* 23(6):1163-1164.

Fan, D.-F., and D. S. Feingold. 1972. Biosynthesis of uridine diphosphate D-xylose. V. UDP-D-galacturonate carboxy-lyase of *Ampullariella digitata*. *Arch. Biochem. Biophys.* 148(2):576-580.

Gilardi, E., L. R. Hill, M. Turri, and L. G. Silvestri. 1960. Quantitative methods in the systematics of *Actinomycetales*. *J. Microbiol.* 8(4):203-218.

Goodfellow, M. 1967. Numerical taxonomy of some named bacterial cultures. *Can. J. Microbiol.* 13:1365-1373.

———. 1971. Numerical taxonomy of some nocardioform bacteria. *J. Gen. Microbiol.* 69:33-80.

Gorisch, H., and F. Lingens. 1972. Properties of chorismate mutase of *Streptomyces venezuelae*. *Arch. Mikrobiol.* 82:147-154.

Gottlieb, D. 1976. Actinomycete taxonomy. Letter to the editor. *Newsl. U.S. Fed. Cult. Coll.* 6(2):5-7.

Hash, J. H., ed. 1975. *Methods in Enzymology*. Vol. 43. Antibiotics. Academic Press, New York. 848 pp.

Hayashibara Co. 1970. Enzymatic preparation of maltose-containing sugar. Netherlands Patent 7,005,070 (March 13).

Heymer, B., Q. Haferkamp, and W. C. Schmick. 1970. Präparation und anwendung FITC-markierter streptokokkenzellwand-kohlenhydrate. *Z. Immunitätsforsch. Allerg. Klin. Immunol.* 140:457-460.

Hill, L. R., M. Turri, E. Gilardi, and L. G. Silvestri. 1961. Quantitative methods in the systematics of *Actinomycetales*. II. *J. Microbiol.* 9:56-72.

Hopwood, D. A. 1973. Developments in Actinomycete genetics. Pages 21-46 *in* Z. Vanek, Z. Hostalek, and J. Cudlin, eds., *Genetics of Industrial Microorganisms*. II. Actinomycetes and Fungi. Elsevier Publishing Co., New York.

Ide, M. 1971. Adenyl cyclase of bacteria. *Arch. Biochem. Biophys.* 144(1):262-268.

Inamine, E., and A. L. Demain. 1970. Location and action of *Streptomyces griseus* α-D-mannosidase, an antibiotic-transforming enzyme. *Biotechnol. Bioeng.* 12(2):159-166.

Jones, G. H., and H. Weissbach. 1970. RNA metabolism in *Streptomyces antibioticus*: Effect of 5-fluorouracil on the appearance of phenoxazinone synthetase. *Arch. Biochem. Biophys.* 137(2):558-573.

Lamanna, C., and M. F. Mallette. 1965. *Basic Bacteriology. Its Biological and Chemical Background*, pp. 43-45. The Williams & Wilkins Co., Baltimore.

Lechevalier, M. P., and H. Lechevalier. 1976. Chemical methods as criteria for the separation of nocardiae from other Actinomycetes. *Biol. Actinomycetes Rel. Org.* 11(2):78-92.

Lehninger, A. L. 1970. *Biochemistry*. Worth Publishers, Inc., New York. 833 pp.

Luedemann, G. M. 1971. Species concepts and criteria in the genus *Micromonospora*. *Trans. N.Y. Acad. Sci.* 33(2):207-218.

Malikova, S., J. Simuth, and J. Zelinka. 1972. Activity of DNA dependent RNA-polymerase in metabolism of *Streptomyces aureofaciens*. *Biologia* (Bratisl.) 27:449-453; *Microbiol. Abstr.* 7B(10):59 Abstract 7B8504 (1972).

Markert, C. L., ed. 1975. *Isozymes*. IV. Genetics and Evolution. Academic Press, New York. 965 pp.

Michal, G. 1974. *Biochemical Pathways*. Rev. ed. Boehringer Mannheim, GMBH, Biochemical Division, West Germany and New York.

Nakamura, S., Y. Marumoto, H. Yamaki, T. Nishimura, N. Tanaka, M. Hamada, M. Ishizuka, T. Takeuchi, and H. Umezawa. 1969. An anti-inflammatory proteinase, kinonase AI and AIII obtained from a *Streptomyces*. *Chem. Pharm. Bull.* (Tokyo) 17:714-721.

Nitsch, B., and H. J. Kutzner. 1969. Egg-yolk agar as a diagnostic medium for streptomycetes. *Experientia* 25:220.

Pridham, T. G. 1976a. Contemporary species concepts in *Actinomycetales*. *In* T. Arai, ed., *Actinomycetales: The Boundary Microorganisms*, Toppan Co., Ltd., Tokyo. In press.

Pridham, T. G. 1976b. Identification of streptomycetes and streptoverticillia at the species level: Revision of 1965 system. *In* T. Arai, ed., *Actinomycetales: The Boundary Microorganisms*. Toppan Co., Ltd., Tokyo. In press.

Rehaček, Z. 1973. Distinction between microorganisms in examination for novelty: Need of generally accepted scientific criteria. Pages 423-433 *in* Z. Vanek, Z. Hostalek, and J. Cudlin, eds., *Genetics of Industrial Microorganisms*. I. Bacteria. Elsevier Publishing Co., New York.

Rogosa, M., M. I. Krichevsky, and R. R. Colwell. 1971. Method for coding data on microbial strains for computers (Edition AB). *Int. J. Syst. Bacteriol.* 21:1A-175A.

Rossi Doria, T. 1891. Su di alcune specie di *"Streptotrix"* trovate nell' aria studiate in rapporto a quelle gia note e specialmente all' *"Actinomyces."* *Ann. Inst. Igiene sper. Univ. Roma* 1:399-438.

Sallach, H. J. 1972. *Intermediary Metabolism*. Chart three of four. P. L. Biochemicals, Inc., Milwaukee, Wisconsin.

Sato, K., S. Shimizu, and S. Fukui. 1971. Studies on the corrinoids and porphyrins in streptomycetes. V. Effect of additions on porphyrin excretion and δ-aminolevulinic acid dehydratase in *Streptomyces olivaceus*. *Agric. Biol. Chem.* 35(3):344-350.

Sears, M. N. 1971. The urease of *Actinoplanes* 1700. *J. Elisha Mitchell Sci. Soc.* 87:175-182.

Silvestri, L., M. Turri, L. R. Hill, and E. Gilardi. 1962. A quantitative approach to the systematics of *Actinomycetales* based on overall similarity. Pages 333-360 *in Microbial Classification*, 12th Symp. for the Society for General Microbiology, Cambridge University Press, Cambridge.

Skyring, G. W., and C. Quadling. 1969. Soil bacteria: Principal component analysis of descriptions of named cultures. *Can. J. Microbiol.* 15:141-158.

Szabó, I., M. Marton, L. Ferenczy, and I. Buti. 1967. Intestinal flora of the larvae of St. Mark's fly. II. Computer analysis of intestinal actinomycetes from the larvae of a bibio-population. *Acta Microbiol. Acad. Sci. Hung.* 14:239-249.

Trejo, W. M. 1970. An evaluation of some concepts and criteria used in the speciation of streptomycetes. *Trans. N.Y. Acad. Sci.* 32(8):989-997.

Tsukumura, M. 1969. Numerial taxonomy of the genus *Nocardia*. *J. Gen. Microbiol.* 56:265-287.
Wayne, L. G. 1967. Selection of characters for an Adansonian analysis of mycobacterial taxonomy. *J. Bacteriol.* 93:1382-1391.
Woodruff, H. B., S. A. Currie, T. C. Hallada, and I. Pulter. 1973. Importance of the description of the producing organisms in obtaining patent protection for fermentation processes. Pages 403-422 *in* Z. Vanek, Z. Hostalek, and J. Cudlin, eds., *Genetics of Industrial Microorganisms*. I. Bacteria. Elsevier Publishing Co., New York.